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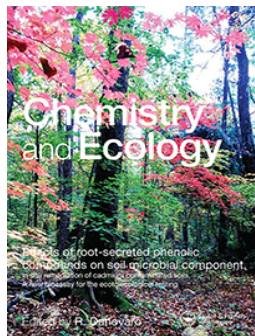
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Some aspects of heavy metals contamination remediation and role of biosurfactants

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Contamination by heavy metals is the result of different industrial activities. The presence of heavy metals in soil and water causes serious problems, as these materials are not biodegradable and do contaminate both biological systems and the subsoil. Biological surface-active compounds otherwise known as biosurfactants in general and rhamnolipids biosurfactants in particular have been successfully employed in the remediation of environments contaminated with heavy metal ions. The aim of the present review is to highlight potential applications of these tensioactive compounds for use in environmental heavy metals removal and bioremediation and processes involved.

Keywords: biosurfactants; heavy metals; remediation

1. Introduction

The term ‘heavy metal’ applies to a group of metal and metalloids with an atomic density greater than 4000 Kg/m³ (four times the density of water). Heavy metals occur naturally in the environment and are found in rocks, soil, plants and animals. Metals occur in different forms as dissolved ions in water or vapour or minerals in rocks, sand and soil. These materials can also bond to organic and inorganic molecules or adhere to particles in the air. Both natural and anthropogenic processes emit metals into the air and water.[1,2]

In the past 300 years, the world population increased from 500 million to > 5.5 billion. This event was accompanied by an increase in pollution levels and the subsequent destruction of a large number of ecosystems, including water resources, which constitute one of the main natural resources affected by environmental pollution. Rivers and oceans commonly receive considerable huge amounts of wastewater containing high concentrations of heavy metals, pesticides and other substances, such as nitrogen, phosphorus and both organic and inorganic compounds.[3] There is a general consensus by most environmentally concerned agencies to reduce the numerous problems caused by large amounts of heavy metals in the water supply

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and efforts are usually directed to diminish the concentration of these elements in industrial wastewater.

The contamination of soil by heavy metals is the result of different industrial activities, such as mining, the production of batteries for vehicles, industrial waste deposits and the dispersal of ash from incineration processes. The presence of heavy metals in soil causes serious problems, as these materials cannot be biodegraded, which leads to the contamination of biological systems and the subsoil due to lixiviation (seepage). In the USA, for example, lead is found in 15% of contaminated lands, followed by chromium, cadmium and copper, which are found in 7–11% of soils.[4,5]

Different technologies have been developed and implanted to reduce costs associated with the treatment of contaminated soil by heavy metals.[6] Two main technologies are normally employed for this purpose. The first consists of immobilising heavy metals in a solid matrix that is strongly bonded to soil, thereby minimising migration. However, this technology is not a definitive solution to the problem when one considers the impossibility of reusing the soil and the need for long-term monitoring. The second technology leads to the mobility of the metal and its migration into the liquid phase by desorption and solubilisation.[7] This technology is considered a more lasting or effective solution by allowing the recycling of the remediated soil and consequent reuse of the land. The most widely employed methods involve cleaning the soil with acids and chelating agents.

Surfactants are amphipathic molecules that constitute a potential solution for the remediation of contaminated soil with metals and oils, as when added into liquid phase solutions they facilitate the solubilisation, dispersal and desorption of contaminants, thereby allowing the reuse of washed soils.[8] Different synthetic surfactants have been evaluated in decontamination tests. However, the need to replace synthetic compounds with natural substances that can achieve similar results has been the object of studies involving the use of microbial biosurfactants.[9]

Due to their diverse structures and properties, biosurfactants have applications in different industrial processes as well as the potential for novel applications. It is believed that biosurfactants will become known as multifunctional materials of the new century.[8] Thus far, the petroleum industry has been the largest market for biosurfactants, as such compounds can be used in the clean-up of oil spills, the removal of residual oil from storage tanks, the advanced recovery of petroleum as well as the bioremediation of contaminated soil and water.[10,11]

The viability of biosurfactants for the removal of metals has been demonstrated some time ago.[12] A number of studies have described the potential of biosurfactants, such as surfactin and rhamnolipids, both of which are of a bacterial origin,[13,14] and other glycolipids originating from yeasts.[15–20] The ionic nature of these compounds as well as their biodegradability, lower degree of toxicity and exceptional surface properties make biosurfactants potential candidates for the removal of heavy metals from soil and sediment.

Considering the serious problems caused by the contamination of heavy metals and the possibility of applying the emerging biosurfactant technology for the remediation of soil, the aim of the present review of the literature was to analyse the state of the art regarding the potential use of biosurfactants in heavy metals removal/remediation.

2. Surfactants/biosurfactants

Surfactants are surface-active molecules with molecular structures containing both hydrophilic and hydrophobic moieties that tend to be distributed at the interfaces between liquid phases with different degrees of polarity (oil/water), causing a reduction in both surface and interfacial tension, which is essential for detergency, emulsification, lubrication, solubilisation and phase

dispersal.[21] They are employed in cleaning products (soaps and detergents), the petroleum industry, cosmetics and hygiene products. The world production of surfactants exceeds three million t per year, the majority of which are used as a raw material in the manufacturing of detergents for household use.[22] Examples of commercially employed ionic surfactants include sulphate esters or fatty acid sulphates (anionic) and quaternary ammonium salts (cationic).[7,13]

2.1. Properties

The most important property of a surfactant is the reduction in surface tension, which is the force of attraction between molecules in liquids.[22] Surface is defined as the limit between a liquid and the air and interface is defined as the limit between two liquids. Thus, the tension between air/water and oil/water phases is known as surface tension and interfacial tension, respectively.[21]

Biosurfactants are surfactants produced by biological systems mainly microorganisms and vary widely in chemical structure and characteristics.[2,23] Despite the diversity in chemical composition and properties, most biosurfactants have some common characteristics that offer advantages over conventional surfactants [24] which include:

- Surface and interfacial activity: Biosurfactants are more efficient and effective than conventional surfactants due to lower surface tension values at lower concentrations. The Critical Micelle Concentration (CMC) of biosurfactants, which is a measure of the efficiency of these natural compounds, ranges from 1 to 2000 mg/L, whereas interfacial tension (oil/water) and surface tension are around 1 and 30 mN/m, respectively.
- Tolerance to temperature, pH and ionic force: Many biosurfactants can be used under extreme conditions and retain activity after sterilisation. For instance, glycolipids are known to be unaffected by sterilisation at 121°C and pH ranging from 5 to 12. Many biosurfactants tolerate salt concentrations up to 10%, whereas a salt concentration of > 2% can inactivate most conventional surfactants.
- Biodegradability: Biosurfactants are easily degraded by bacteria and other microorganisms in water and soil, which makes these compounds suitable for bioremediation applications and waste treatment.
- Low toxicity: Biosurfactants have received greater attention due to the growing concern on the part of the population regarding the allergic effects of artificial products. Moreover, the low degree of toxicity allows the use of these compounds in food, cosmetic and pharmaceutical products.
- Availability: Biosurfactants can be produced from widely available raw materials, including industrial waste, as sustainable substrate.
- Specificity: As complex organic molecules with specific functional groups, biosurfactants also exhibit specificity, which is of considerable interest in the detoxification of specific pollutants or particular applications in the foods, cosmetics and pharmaceuticals.
- Biocompatibility and digestibility: Such properties ensure the safe use of these biomolecules in the food, cosmetic and pharmaceutical industries.

Despite the many advantages biosurfactants offer, there are some challenges that remain to be addressed which include the following:

- The large-scale production of biosurfactants can be costly. However, this problem could be solved with a combination of low-cost substrates.[25]
- The acquisition of products with a high degree of purity is difficult due to the need for consecutive broth purification steps or downstream processing.[26]

- Super-producing species are rare and those that are known are incapable of producing high surfactant yields. Moreover, such microorganisms require complex culture media.[27]
- The regulation of biosurfactant synthesis is not fully understood, as these biomolecules can be primary or secondary metabolites and mostly under complex quorum-sensing control mechanisms.[11]
- The increase in productivity is often compromised by the formation of foam, which requires the use of diluted media or costly collection technologies.[21]

2.2. Classification

Synthetic surfactants are classified based on the ionic charge in the polar part of the molecule. In the presence or absence of electrical charges, conventional surfactants can be anionic, cationic, non-anionic or amphoteric.[28] Most biosurfactants are anionic or neutral. Only a few are cationic, such as those that contain amine groups. The hydrophobic moieties are mostly characterised by long-chain fatty acids, whereas the hydrophobic moiety may be carbohydrate, amino acid, cyclic peptide, phosphate, carboxyl acid or alcohol. Biosurfactants are commonly classified based on their biochemical nature or the producing microbial species. With regard to structure, these natural compounds are classified into five major groups [29]:

- Glycolipids, the degree of polarity of which depends on the hydrocarbons employed as substrates. Rhamnolipids produced by *Pseudomonas aeruginosa* and sophorolipids produced by species of *Candida* are examples of glycolipids.
- Liposaccharides, which normally have a high molar mass and are soluble in water, such as Emulsan, which is an extracellular emulsifier produced by the bacterium *Acinetobacter calcoaceticus* using a hydrocarbon substrate.
- Lipopeptides, such as surfactin, which is produced by *Bacillus subtilis* and is one of the most potent biosurfactants reported in the literature thus far.
- Phospholipids, which are common to a large number of microorganisms, such as the biosurfactant from *Corynebacterium lepus*.
- Fatty acids and neutral lipids, some of which are classified as glycolipids, and hydrophobic proteins.

2.3. Biosurfactant-producing microorganisms

A large variety of microorganisms (bacteria, yeast and filamentous fungi) are capable of producing biosurfactants with different molecular structures, as shown in Table 1.

2.4. Biosurfactant production using industrial waste as substrates

Most known biosurfactants are produced on carbon substrates that are insoluble in water, such as solid and liquid hydrocarbons, oils and fats, although many have been obtained using soluble substrates.[7,22] The availability and type of raw material considerably affect the production cost. It is estimated that the raw material accounts for 10–30% of the total cost of a biotechnological product.[30] Moreover, huge amounts of polluting waste products are discarded every year throughout the world. The treatment and removal of this waste represent a high cost for different industries and a significant interest has been demonstrated of using some of these wastes in biosurfactant production.[31] Indeed, many biosurfactants have been produced using renewable, low-cost, agro-industrial substrates. Vegetable oils, waste vegetable fry oils, residue from

Table 1. Main classes of biosurfactants and respective producing microorganisms modified from Silva et al. [2].

Class/type of biosurfactant	Microorganisms
<i>Glycolipids</i>	
Rhamnolipids	<i>P. aeruginosa</i> , <i>Burkholderia</i> sp.
Sophorolipids	<i>Candida bombicola</i> , <i>C. apicola</i>
Trehalolipids	<i>Rhodococcus erythropolis</i>
Mannosylerythritol lipids	<i>Candida antarctica</i> , <i>Pseudozyma</i> sp. and <i>Ustilago</i> sp.
Glycolipids	<i>Burkholderia cenocepacia</i>
<i>Lipopeptides and lipoproteins</i>	
Lichenysin	<i>Bacillus licheniformis</i>
Viscosin	<i>Pseudomonas fluorescens</i>
Serrawettin	<i>Serratia marcescens</i>
Surfactin	<i>B. subtilis</i>
Subtilisin	<i>B. subtilis</i>
Gramicidin	<i>Bacillus brevis</i>
Polymyxin	<i>Bacillus polymyxa</i>
<i>Fatty acids, neutral lipids and phospholipids</i>	
Fatty acids	<i>C. lepus</i>
Spiculisporic acid	<i>Penicillium spiculisporum</i>
Neutral lipids	<i>Nocardia erythropolis</i>
Phospholipids	<i>Thiobacillus thiooxidans</i>
Phosphatidylethanolamine	<i>Acinetobacter</i> sp., <i>R. erythropolis</i>
<i>Polymeric surfactants</i>	
Emulsan	<i>A. calcoaceticus</i>
Alasan	<i>Acinetobacter radioresistens</i>
Biodispersan	<i>A. calcoaceticus</i>
Liposan	<i>C. lipolytica</i>
Carbohydrate–lipid–protein	<i>Pseudomonas fluorescens</i>
Mannan–lipid–protein	<i>Candida tropicalis</i>
<i>Particulate surfactants</i>	
Vesicles	<i>A. calcoaceticus</i>
Whole microbial cells	<i>Cyanobacteria</i>

oil distilleries, by-products of the milk industry (whey), sugarcane molasses and glycerine have been cited in the literature.[23]

2.5. Environmental considerations

Heavy metals are natural components of the crust of the earth many of which act as essential micro-nutrients for living beings, although at high concentration can lead to severe intoxication.[32] The most toxic forms of heavy metals in their ionic species are more stable oxidation states, such as Cd^{2+} , Pb^{2+} , Hg^{2+} , Ag^{+} and As^{3+} , which react with biological molecules in the body to form extremely stable biotoxic compounds that are difficult to dissociate. In the environment, heavy metals are generally more persistent than organic contaminants, such as pesticides and petroleum by-products. Such metals are not biodegradable and can be transferred from one chemical state to another, which changes their mobility and toxicity. Depending on pH and speciation, these substances can become mobile in soil. Table 2 describes the chemistry and speciation of some heavy metals. Thus, different fraction may be lixiviated to groundwater or become bioavailable to living organisms.[44]

The accumulation of heavy metals in European and American soils reaches 939,000 t of copper, 783,000 t of lead, 1,372,000 t of zinc and 22,000 t of cadmium.[3] Some developing countries such as Brazil have no adequate legislation regarding the maximum heavy metal levels permitted and high rates of industrial contamination with these pollutants have been frequently

Table 2. Chemistry and speciation of some heavy metals.[33]

Metal	Speciation and chemistry	Concentration limits	References
Lead	Occurs in oxidation states 0 and +2. Pb(II) is the most common/reactive form. Has reduced solubility upon complexation with inorganics (Cl^- , CO_3^{2-} , SO_4^{2-} , PO_4^{3-}) and organic ligands (humic acid, EDTA, amino acids). Fixation in soil occurs through absorption, ionic exchange, precipitation and complexation with sorbed organic matter	Agricultural soil: 7–20 ppm; Levels in soil: up to 300 ppm; Limit in drinking water according to US EPA: 0.015 ppm	[34–37]
Chromium	Occurs in oxidation states +6 and +3. Cr(VI) is the toxic form of Cr in groundwater. Most species of Cr(VI) include chromate (CrO_4^{2-}) and dichromate ($\text{Cr}_2\text{O}_7^{2-}$), especially Ba^{2+} , Pb^{2+} and Ag^+ . Cr(III) is dominant at pH < 4. Cr(VI) can be reduced to Cr(III) by organic matter, S^{2-} and Fe^{2+} ions under anaerobic conditions; Lixiviation increases with pH increase	Normal concentration in subsoil: < 0.001 ppm; Lethal dose: 1–2 g; Limit in drinking water according to US EPA: 0.1 ppm	[38,39]
Zinc	Occurs in oxidation states 0 and +2 and forms complexes with anions, amino acids and organic acids. Zn is available at high pH values. Hydrolyses at pH 7.0–7.5, forming $\text{Zn}(\text{OH})_2$. Precipitates under reduced conditions and co-precipitate with Fe and Mn hydroxides.	Natural concentration of Zn in soil: 30–150 ppm; Concentration in plants: 10–150 ppm; Toxicity in plants: 400 ppm; Limit in water established by WHO: 5 ppm	[35,38,39]
Cadmium	Occurs in oxidation states 0 and +2. Hydroxides ($\text{Cd}(\text{OH})_2$) and carbonates (CdCO_3) predominate at high pH. Aqueous sulphate species predominate at pH < 8. Cd precipitates in the presence of phosphate, arsenic, chromate, sulphide and is mobile in the pH range from 4.5 to 5.5	Natural concentration of Cd in soil: > 1 ppm; In plants: 0.005–0.02 ppm; Toxicity in plants: 5–30 ppm; Limit in water according to US EPA: 0.005 ppm	[39,40]
Arsenic	Occurs in oxidation states –3, 0, +3 and +5. As(V), dominant in anaerobic environments, usually as arsenate (AsO_4^{3-}). Behaves as a chelating agent and can co-precipitate with or adsorb to iron hydroxides under acidic conditions. As(III) dominates under reduced conditions as arsenite (AsO_3^{3-}), soluble in water and can co-precipitate with or adsorb to metal sulphates	Limit in drinking water according to US EPA and WHO: 0.01 ppm	[34,39]
Iron	Occurs in oxidation states 0, +2, +3 and +6. Organometallic compounds contain oxidation states +1, 0, –1 and –2. Fe(IV) is a common intermediate agent in many biochemical oxidation reactions. A large number of compounds with various valences contain both Fe(II) and Fe(III) centres, such as magnetite and Prussian blue	Maximum tolerable ingestion level: Adults: 45 mg/day; Children: 40 mg/day	[41]

(Continued)

Table 2. Continued.

Metal	Speciation and chemistry	Concentration limits	References
Mercury	Occurs in oxidation states 0, +1 and +2. Can occur in the alkylated form (methyl/ethyl mercury), depending on conductivity and pH of the system. Hg^{2+} and Hg_2^{2+} are more stable under oxidant conditions. Sorption in soil, sediment and humic matter increases with the increase in pH	Natural concentration in soil: > 0.0002 ppm; Limit in drinking water according to US EPA: 0.002 ppm	[34,39]
Copper	Occurs in oxidation states 0, +1 and +2. Cu^{2+} ions, $\text{Cu}(\text{OH})^+$ and $\text{Cu}_2(\text{OH})_2^{2+}$ are the most toxic species. CuCO_3 is the dominant soluble species in alkaline aerobic systems. $\text{CuS}(\text{s})$ forms in the presence of sulphur in anaerobic environments. Cu forms strong complexes with humic acids	Natural concentration in soil: 2–100 ppm; Normal concentration in plants: 5–30 ppm; Toxic concentration in plants: 30–100 ppm; Limit in water according to US EPA: 1.3 ppm	[42,43]

recorded. In recent years, the Ingá Mercantile an industrial company, which was a zinc-producing factory located 85 km from the city of Rio de Janeiro and which has been deactivated nearly 15 years ago, has been transformed into the largest area of toxic waste contamination. Heavy metals, such as zinc, cadmium, mercury and lead, continue to pollute the soil and water, affecting the mangrove environment and human population. This has occurred due to the fact that the channels originally built to contain the contaminated area and water have not been properly maintained for many years and therefore, the nearby lands have become frequently flooded contaminating the mangrove vegetation.

Another case occurred in the city of Bauru, state of São Paulo, Brazil, in 1999. The Ajax Accumulator Industry, which is one of the largest automotive battery manufacturers in the county, was reported to have contaminated 113 children through the lead expelled from its chimneys at rates greater than 10 milligrams/decilitre. The city of Paulínia, also in the state of São Paulo, was contaminated by Shell Chemical of Brazil. Among the 166 residents who were examined, 53% exhibited chronic contamination and 56% of the children had high concentrations of copper, zinc, aluminium, cadmium, arsenic and manganese in their systems.[43] Moreover, the incidence of liver tumours, thyroid tumours, neurological disorder, dermatitis, allergic rhinitis as well as gastrointestinal, pulmonary and hepatic disorders have all been recently reported.[43]

3. Heavy metal treatment technologies

In recent decades, a large number of remediation technologies have been used to treat contaminated soil and groundwater throughout the world and many documents are available on technologies for the remediation of organic and inorganic pollutants.[12,45,46] Review articles on the removal of heavy metals from wastewater were also published.[37] In addition details on heavy metal remediation technologies for groundwater were published by the US Environmental Protection Agency.[45] Application technologies under certain operational conditions are limited due to the fact that such technologies may not function effectively under other conditions.

Groundwater is wholly related to the soil through which it flows. In the past, some technologies were applied only to remove petroleum products, whereas others were employed for the removal of heavy metals. Over the years, this barrier has been overcome as researchers throughout the world often combined different technologies to achieve the desired results. Heavy metal removal

technologies have three categories: (1) chemical; (2) biological/biochemical/biosorptive and (3) physicochemical. In some cases, these technologies overlap as a result of the new paradigms in science and technology, with interdisciplinary studies gaining ground in compartmentalised studies.[33]

3.1. Chemical treatment technologies

Groundwater contaminants are often dispersed in plumes over large areas just below the surface, which hinders the use of conventional remediation technologies. In such cases, chemical treatment may be the best choice to reduce the contamination by converting these metals into inactive states through oxidation/reduction neutralisation reactions.[46] Reducers, such as dithionite, colloidal iron (Fe 0 and Fe II) and hydrogen sulphite gas, can be injected into the contaminated area, but alkaline pH and a high degree of soil permeability are prerequisites. However, toxic intermediate agents may be produced that may negatively affect the process. Chemical cleaning is usually an effective permanent solution as it allows the recycling of the remediated soil and consequent reuse of the land. Acids and chelating agents, such as ethylenediaminetetraacetic acid (EDTA) are the most widely employed for cleaning soil; however, acids reduce soil fertility and alter its physicochemical composition due to the dissolution of minerals.[47] EDTA is also carcinogenic in nature and is an expensive product. The difficulty in recovering the metal from the metal–chelating agent complex also restricts the use of this method.[33,48]

3.2. Physicochemical treatment technologies

Physicochemical methods depend on physical processes or activities, such as barrier construction, physical adsorption or absorption, mass transference and biochemical processes. Most often, two or more processes are used for the treatment of contamination. Permeable reactive barriers (PRBs) are the most practical solution among groundwater remediation methods. Such barriers, however, are prone to clogging, reduction in permeability and deviation in the flow of groundwater. The breakdown of a PRB also hinders its activity and reloading, leading to the need for replacement or repositioning, which are a major challenge that requires constant monitoring to ensure proper performance. This technology mainly relies on sorption, precipitation, reduction and biological processes.

3.3. Biological, biochemical and biosorptive treatment technologies

Natural biological activity is not capable of removing heavy metals from deep soil layers or groundwater. However, biological processes, such as a phytoremediation and bioremediation (use of microorganisms), can be employed for long-term restoration in conjunction with any other, more intensive restoration process. Microorganisms can exert an influence on metals in different ways. Some forms of metals can be transformed through redox processes or alkylation. Metals can also be accumulated by microorganisms through independent metabolic ingestion or dependent metabolic absorption. Microorganisms can exert an indirect influence on the mobility of a metal by affecting the pH or through the production or release of substances.[49] Genetically modified organisms can also play a more active role in this process.

Enhanced bioremediation is an actively researched field. The immobilisation of radionuclides, such as U, Tc and Ra, by microorganisms of the genus *Geobacter* is a very recent method. Biobarriers can be used to remediate such radionuclides in groundwater. The *in situ* bioprecipitation process immobilises heavy metals, such as sulphite precipitates, through the biological reduction of sulphates, but the stability of sulphites under redox conditions and following

a change in pH continues to be a debatable issue. The sulphate reduction process involves a broad choice of electron donors to enhance the activity of sulphate-reducing bacteria and can be applied to a reactive barrier or an *ex situ* anaerobic bioreactor, but is a quite difficult task. Acidic draining from mines can be effectively treated with the biological reduction of sulphates.[49]

The *in situ* remediation of arsenic and ferrous oxides by microorganisms has proven to be a very effective and sustainable technology. This is a long-term process with a long-lasting effect on an aquifer. No waste is generated and virtually no chemical product is needed to create an oxygenation zone in the aquifer. The process maintains an adequate balance of co-precipitation between As(V) and Fe(III). Biosorption is a very practical solution for the remediation of heavy metals and had been widely investigated as a method for removing metals from soil and groundwater. However, the application of this method in the field remains limited. The absorption of metals by different microorganisms is the slowest natural processes that can be used in the field for measures of long-lasting restoration. This method can also be applied in fluidised bed reactors. However, immobilised metals can be lixiviated back to the solution under acidic conditions. Agricultural waste and cellulosic materials have enormous potential for use regarding the biosorption of heavy metals through the ionic exchange process, complexation and electrostatic interactions. Simple pre-treatments involving chemical agents may be necessary to enhance sorption power and stability. The low cost, lack of toxicity, high adsorption rate and ample availability of sportive materials have made investigating this technology option more lucrative and attractive.[50–52]

4. Removal of heavy metals by biosurfactants

The aim of using biosurfactants in the decontamination of organic compounds, such as hydrocarbons, is to increase their bioavailability or mobilise and remove the contaminants through pseudo-solubilisation and emulsification during a cleaning treatment.[53] The use of biosurfactants in the recovery of inorganic compounds involves chelating action and the removal of such ions during the cleaning step, which is facilitated by chemical interactions between amphipathic compounds and metal ions.[21]

Biosurfactants use in heavy metals remediation has undisputable advantages, as microorganisms capable of producing surfactant compounds do not need to have survival capacity in contaminated soil with heavy metals, although biosurfactants require the continuous addition of new portions of these compounds.[22] Biosurfactants facilitate the solubilisation, dispersal and desorption of contaminants in soil, thereby allowing the reuse of the land.[12]

Two main approaches are involved in the remediation of contaminated soil with heavy metals. The first is employed *ex situ* – the contaminated soil is excavated, placed in a glass column and washed with a biotensioactive solution. The second consists of the *in situ* washing of the soil and involves the use of drainage tubes and trenches for the introduction and collection of the biosurfactant solution. Biosurfactants can be applied to a small portion of contaminated soil. The soil is placed in an enormous cement mixer and the biosurfactant metal complex is removed. The soil is then deposited back into the ground and the biosurfactant–metal complex is treated to precipitate the biosurfactant, leaving the metal behind. The bond formed between the positively charged metal and negatively charged tensioactive agent is usually strong enough that the water washing through the soil removes the metal–surfactant complex.

4.1. Mechanisms of heavy metal removal by biosurfactants

The use of biosurfactants for the bioremediation of heavy metals from contaminated soil is mainly based on its capacity to form complexes with metals. Anionic biosurfactants create

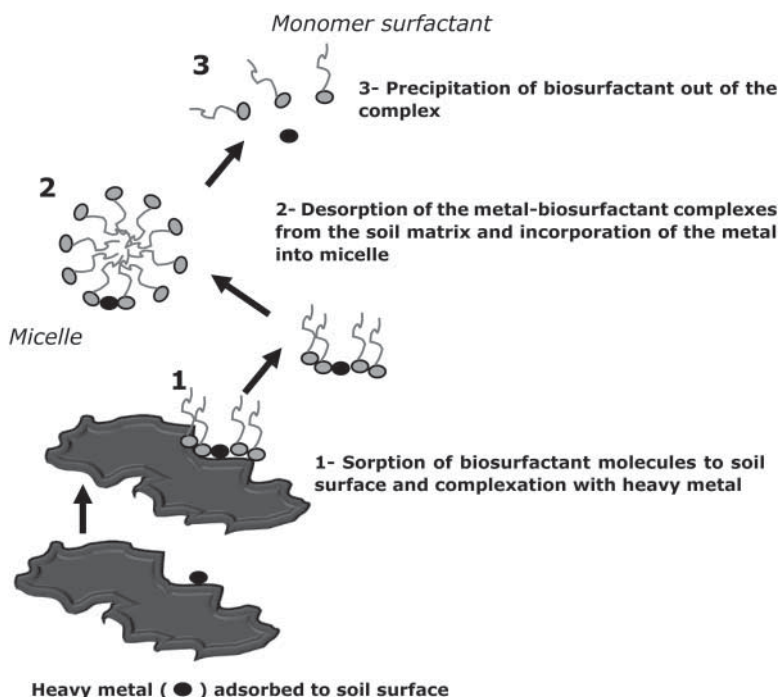


Figure 1. Schematic diagram showing the mechanism of removal of heavy metals by ionic biosurfactants.

complexes with metals through ionic bonds, which are stronger than the bond between the metal and soil. The metal–biosurfactant complex is then desorbed from the soil matrix to the soil solution due to the reduction in interfacial tension. Cationic biosurfactants can replace the same charged ions through competition with some, but not all negatively charged surfaces (ion exchange). Metal ions can also be removed from the soil by biosurfactant micelles.[22,54–57,65]

The removal of metals by ionic biosurfactants occurs in the following sequence: (1) sorption of the biosurfactant to the soil surface and complexation with the metal; (2) detachment of the metal to the solution and (3) adsorption to the micelles of the biosurfactant. Heavy metals become attached to the micelles through electrostatic interactions as illustrated in Figure 1 and are easily recovered using membrane separation methods.[59]

Different synthetic surfactants have been evaluated in decontamination tests.[60] However, the need to replace synthetic compounds with natural counterparts has led to research on the use of biosurfactants. In recent years, a number of biosurfactants have been proposed for the bioremediation of soil contaminated with heavy metals and studies have demonstrated the potential of these biological agents, such as rhamnolipids and surfactin, which are of a bacterial origin, as well as sophorolipids produced by yeasts.

4.1.1. Removal of heavy metals by rhamnolipids

Rhamnolipids are the group of biosurfactants most cited in the literature for application in heavy metals removal.

Juwarkar et al. [55] investigated the removal of cadmium and lead by a biosurfactant produced by *P. aeruginosa* BS2, performing experiments in columns for the determination of the removal of the heavy metals by a rhamnolipid, which removed more than 92% of the cadmium and 88% of the lead within 36 hours at a concentration of 0.1%. Rhamnolipids were also able to reduce

toxicity and allow microbial activity (*Azotobacter* and *Rhizobium*), thereby avoiding the decline in the soil integrity. Wang and Mulligan [65] evaluated the viability of rhamnolipid foam for the removal of Cd and Ni from sandy soil and report a significant effect on the mobility of the biotensioactive agent, which flowed through a porous medium. The rhamnolipid foam increased efficiency and allowed the removal of 73.2% and 68.1% of Cd(II) and Ni, respectively, whereas the rhamnolipid solution only removed 61.7% and 51%, respectively. The heavy metal removal rate from soil depends strongly on its chemical composition.

As the sorption of a biosurfactant can reduce its potential for the removal of contaminants from different components of the soil, [16] tests were carried out on the sorption of rhamnolipids, which reduced its removal efficiency, demonstrating the dependence on the concentration of the surfactant. Asci et al. [60] evaluated the removal potential of Cd(II) from kaolinite. Different sorption models were evaluated. The desorption effects of pH and rhamnolipid concentration were evaluated and sorbed Cd(II) was determined. Optimum pH was 6.8 and the optimum concentration of the rhamnolipid was 80 mM, allowing the removal of 71.9% of Cd(II). Asçi et al. [9] and Asçi et al. [56] investigated zinc removal from feldspar (a component of the soil) using rhamnolipids and reported a significant reduction in the heavy metal. Optimum pH was 6.8 for removal due to the formation of micelles under this condition. Interfacial tension was low in this range, which facilitates contact between the metal and biosurfactant and subsequent sorption of the metal. The optimum concentration was 25 mM for the removal of 2.2 mM of zinc and the removal rate was 98.8%.

Dahrazma and Mulligan [15] evaluated the performance of a rhamnolipid in a continuous flow configuration for the removal of heavy metals (copper, zinc and nickel) from sediment removed from the Lachine Canal in Canada. The rhamnolipids solution was pumped through the sample in a sediment column at a constant flow. The removal rates using rhamnolipids were 37% for Cu, 13% for Zn and 27% for Ni. The addition of NaOH at a concentration of 1% to the rhamnolipids at a concentration of 0.5% enhanced copper removal fourfold in comparison to rhamnolipids alone. Many metals mainly exist in the organic portion of the environment and the addition of OH⁻ in sediments solubilises this portion. Thus, more metals are available for removal by the biotensioactive rhamnolipids. This information is valuable to the creation of a remediation protocol for the cleaning of sediment. Dahrazma et al. [61] evaluated the micelles of a rhamnolipid and found that pH had a significant effect on morphology. At a high pH, large aggregates of micelles on the order of 17 Å were found. Under acid conditions, however, vesicles with a diameter of 500–600 Å were formed. Thus, there must be no filtering effect during the washing of soil through pores, which are typically 200 nm in size. The complexation of micelles with metals, therefore, demonstrated no significant effect on micelle size.

Massara et al. [62] described a new approach to the stabilisation of metals by biosurfactants. Hexavalent chromium [Cr(VI)] is a toxic environmental pollutant which can be generated by the oxidation of its trivalent form [Cr(III)] under specific conditions. A study was conducted on the removal of Cr(III) to remove the danger of its oxidation. The effect of the addition of biosurfactants with a negative charge (rhamnolipids) was investigated in kaolinite contaminated with chromium. Rhamnolipids demonstrated the ability to extract 25% of the stable form of chromium [Cr(III)] under optimum conditions. The removal of Cr VI was also enhanced twofold when using rhamnolipids containing solution. The results of the sequential extraction process demonstrated that rhamnolipids removed Cr(III) mainly from the carbon oxide and hydroxide portions of kaolinite. Rhamnolipids also exhibited the ability to remove 100% of Cr(VI) and Cr(III) throughout a 24-hour period, demonstrating that these biosurfactants can be beneficial to the removal or conversion of Cr(VI) to Cr(III) in the long term. Ara and Mulligan [63] also evaluate the viability of the use of rhamnolipids for the removal and reduction of Cr(VI) from contaminated soil and water. The initial chromium and rhamnolipids concentrations, pH and temperature all affected the efficiency of the reduction. Rhamnolipids were able to reduce the

initial Cr(VI) in water by 100% when present at low concentration (10 ppm) and under optimum conditions (pH 6, 2% rhamnolipid concentration and 25°C). At higher initial Cr(VI) concentrations (400 ppm), 24 hours were required to reduce Cr by 24.4%. In soil, rhamnolipids were only capable of removing the soluble portion of Cr. Extraction was enhanced with the increase in the initial concentration in the soil, but diminished slightly with the increase in temperature above 30°C.

The tendency to reduce the extracted chromium was the same as in the aqueous medium. A sequential extraction study was carried out on the soil before and after cleaning to identify the portion of soil that rhamnolipids removed the chromium from. Exchangeable and carbon fractions accounted for 24% and 10% removal of total Cr present, respectively. Oxides and hydroxide retained 44% of the chromium, whereas organic and residuals fractions accounted for 10% and 12%, respectively. Rhamnolipids were able to remove most of the Cr from the exchangeable (96%) and carbon (90%) portions and some from the oxide and hydroxide (22%) portion, but were unable to remove this heavy metal from the other fractions. This information is important to the establishment of the appropriate soil-cleaning processes.[63]

Juwarkar et al. [1] confirmed rhamnolipids abilities to mobilise and decontaminate heavy metals-contaminated soils. The authors used a di-rhamnolipid biosurfactant, which is one of the main congeners of rhamnolipids produced by *P. aeruginosa* BS2, in a glass column study at a concentration of 0.1% to remove chromium, lead, cadmium and copper. They reported that the di-rhamnolipid selectively removed the heavy metals in the following order: Cd = Cr > Pb = Cu > Ni. Wen et al. [64] studied the degradation of a rhamnolipid in soil contaminated with Cd(II) and Zn(II) and found that the biosurfactant was able to remain in the soil long enough to enhance the phyto-extraction of the metals. The ability of biosurfactants to mobilise arsenic from mining waste has also been investigated.[58,66] The experiments demonstrated that the introduction of a rhamnolipid significantly enhanced the mobilisation of this waste. Mobilisation increased with the increase in the concentration of the biotensioactive agent and became relatively stable when the concentration of the rhamnolipid surpassed the CMC concentration which for rhamnolipids varies between 30 and 100 mg/L. The higher concentration of the rhamnolipid needed in some experiment may be due to the sorption of the biosurfactant to the mining waste as well as the dilution of and bonding to waste particles or impurities in the product itself.

Biosurfactants are usually able to enhance the mobilisation of As by reducing the interfacial tension between this heavy metal and the mining waste through the formation of aqueous complexes or micelles, thereby increasing the wettability of the waste. Besides mobilisation, biosurfactants may be involved in other processes related to the recovery of heavy metals. For instance, these natural compounds have been used to entrap trivalent chromium in micelles, thereby enhancing bacterial tolerance and resistance to high concentrations of Cr(III). Asci et al. [67] described the use of rhamnolipids for the extraction of Cd(II) and Zn(II) from quartz. When 0.31 mMol/Kg of Cd(II) in quartz was treated with 25 mM of rhamnolipid, 91.6% of the sorbed Cd(II) was recovered and 87.2% of Zn(II) was extracted using the rhamnolipid at the same concentration. On average, 66.5% of Zn (II) and 30.3% of Cd (II) were released with high charges or the saturation of metal ions in quartz, indicating that a relatively large portion of metal ions was irreversibly retained in the quartz.

Slizovskiy et al. [68] studied the remediation of soil contaminated with heavy metals enhanced by a cationic surfactant (DPC), a non-ionic surfactant (mmonyxKP) and an ionic rhamnolipids biosurfactant (JBR-425), the latter of which achieved the best elution of Zn (39%), Cu (56%), Pb (68%) and Cd (43%). Huang and Liu [69] suggested that the biosurfactant-producing *Pseudomonas* sp., rather than its purified product, may be used to remove Cd and Pb from industrial wastewater. In this case, biomass would act as a biosorbent, to which both physical and chemical sorption can take place simultaneously.

4.1.2. Removal of heavy metals by other biosurfactants

Lipopolysaccharides (LPS) are one of the main types of biosurfactant that are composed of a hydrophilic polysaccharide moiety and hydrophobic phospholipids. They were first tested for the extraction of heavy metals by Langley and Beveridge [70] who demonstrated that LPS increased the hydrophilicity of the outer cell walls, thereby facilitating the absorption of metallic cations by bacterial cells. Kim and Vipulanandan [71] evaluated the removal of lead from water and contaminated soil (kaolinite). The biotensioactive agent was produced by *Flavobacterium* sp. grown on used vegetable oil. More than 75% of the lead was removed from 100 mg/L of contaminated water at 10 times the CMC.

Das et al. [72] investigated the use of a biotensioactive agent produced by a marine bacterium for the removal of heavy metals from solutions. The researchers found that the removal of cadmium from the aqueous solution also occurred at concentrations lower than the CMC and that a concentration of five times the CMC resulted in the nearly complete removal of 100 ppm metal ions. The removal of heavy metals from sediment was enhanced by the use of a solution containing the biotensioactive agent and inorganic compounds. Gnanamani et al. [73] studied the bioremediation of Cr(VI) using a lipopeptide biosurfactant produced by *Bacillus* sp. MTCC 5514. Remediation involved two processes: the reduction of Cr(VI) to Cr(III) through extracellular chromoreductase and the entrapment of Cr(III) by the biosurfactant. The first process transformed the toxic state of chromium into a less toxic state and the second avoided the exposure of the bacterial cells to Cr(III). Both reactions maintained the bacterial cells active throughout the entire experiment and promoted tolerance and resistance to high concentrations of both forms of chromium.

Lipopeptidic biosurfactants have also been exploited as ion collectors in wastewater treatment using a foam flotation process. This two-stage technique is based on the application of a surface-active material or compound to adsorb the metals from the water and a subsequent separation by flotation of the resulting foam.[74] Such a method has been applied to different metals and by using different biosurfactants. For example, Zouboulis et al. [75] investigated the removal of Zn and Cr ions from aqueous solutions. They concluded that the application of the biosurfactants Surfactin-105 and Lichenysin-A as flotation collectors for the separation of the metal-loaded sorbents resulted in better float abilities of metal-laden sorbents compared with chemically produced surface-active compounds such as sodium dodecyl sulfate (SDS) or dodecylamine. Similar results were obtained by Chen et al. [76], who observed a higher Hg removal from artificially contaminated water with surfactin than with SDS and Tween-80, when all were used at a concentration of $10 \times \text{CMC}$.

Other types of biosurfactants (mostly sophorolipid in nature) produced by species of the genus *Candida* have also been successfully employed in the flotation of heavy metals and have demonstrated the capacity to remove more than 90% of cations in columns and air-dissolved flotation processes.[18,19] Biosurfactant produced by the yeast *Candida lipolytica* was also used for the removal of heavy metals and petroleum derivatives using a soil barrier. Biosurfactant significantly reduced soil permeability, demonstrating its applicability as an additive in reactive barriers allowing the removal of around 96% Zn and Cu and the reduction of Pb and Cd concentrations in groundwater.[77,78]

Biosurfactants from plant origin have also been successfully applied in the removal of heavy metals. The use of plant-based biosurfactant saponin was evaluated for the removal of heavy metals from soil at a construction site and sediment from a lake in Japan.[79] The soil contained 890 mg/Kg of zinc, 260 mg/Kg of copper, 170 mg/Kg of nickel and 230 mg/Kg of total petroleum hydrocarbons. The sediment contained 4440 mg/Kg of zinc, 94 mg/Kg of copper and 474 mg/Kg of lead. The highest removal rates (88% for zinc at pH 3 and 76% for nickel at pH 5) were obtained after five washings of the soil with saponin (30 g/L). Using the sediment, the

highest zinc and lead removal rates of 33% and 24%, respectively were achieved with 30 g/L, which is quite a high concentration. The sequential extraction demonstrated that the zinc oxide, copper and organic fractions were substantially reduced by the biosurfactant.

Song et al. [80] evaluated saponin for the removal of the mixed contaminants phenanthrene and cadmium from soil. The former was removed by solubilisation and the latter formed a complex with carboxyl groups in the saponin. The removal rates for phenanthrene and cadmium were 87.7% and 76.2%, respectively, demonstrating the possibility of combined removal by the tensioactive agent. Chen et al. [76] found that 2000 mg/L of saponin was able to remove 83% and 85% of copper and nickel, respectively, from soil. Yuan et al. [81] tested the removal of heavy metals with a saponin derived from tea using a flotation process. The biosurfactant functioned as both a collector and foaming agent. The complexed ions absorbed to the surface of the air bubbles. The highest removal rate was found for lead (90%), followed by copper (81%) and cadmium (71%). The increase in ionic force slightly diminished the removal efficiency. The complexation of carboxyl groups and bivalent metal ions was observed. More recently, saponin was found to effectively remove high levels of copper, lead, and zinc from soil using foam fractionation.[82]

5. Concluding remarks

One of the most promising field of technology emerging in the last decade is the biological techniques employing biosurfactants as heavy metal removal tools. The efficiency and success of biosurfactants in facilitating removal of heavy metal contaminants from soil systems, however, depends largely on the soil texture, structure, clay content, predominant clay type, cation exchange capacity, permeability, ionic strength, etc. The economics of this process will need to be determined to compare with existing technologies. However, with more development, the use of biosurfactants can be an effective, non-toxic process of remediating dredged sediments contaminated with heavy metals. For this purpose, much work remains needed to optimise biological and engineering processes. Although the literature reports a large number of biosurfactant-producing microorganisms, studies on the enhancement of production have mainly concentrated on few genera, such as *Bacillus*, *Pseudomonas* and *Candida*. As demonstrated in the present review, biosurfactants are candidates for the replacement of synthetic surfactants, especially in the petroleum industry. Thus, greater investments are needed in strategies aimed at optimising cost-effective and viable large-scale production, downstream processing and utilisation of biosurfactants.

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